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Integrative taxonomic evidence for a new species of the short-legged toads *Brachytarsophrys* (Anura, Megophryidae) from Guangxi, China

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Abstract

Currently, the genus *Brachytarsophrys* contains nine species. In this study, we describe a new species of *Brachytarsophrys*, named *Brachytarsophrys guilinensis* **sp. nov.**, from northeastern Guangxi, China, based on morphological, molecular, and acoustic evidence. This new species can be distinguished from other known congeners by the combination of the following morphological characters: (1) smaller body size (SVL 70.0–81.9 mm in eight adult males); (2) head wider than long, with head width nearly 1.5 times the length and about half of the SVL; (3) tongue broad and round, feebly notched; (4) male with nuptial pad on the dorsal surface of the first and second fingers; (5) hind limbs relatively short and robust, heels not meeting when legs are positioned at a right angle to the body; (6) tibiotarsal articulation reaching the angle of the mouth when hind limbs are extended forward alongside the body; (7) absence of outer metatarsal tubercle, with the inner metatarsal tubercle oval and approximately equal to the first toe; (8) fingers without webbing; toes with incomplete webbing, webbing formula: I1 - 2II1 - 2III1½ - 3-IV3- - 1½V; (9) lateral fringes on toes wide; (10) several warts on the outer side of the upper eyelid, with one being relatively larger. Phylogenetic analyses based on mitochondrial 16S rRNA, COI, and Cytb genes revealed that this new species is more closely related to *B. popei* and *B. orientalis* than to other known species, with strong Bayesian posterior probability and moderate bootstrap support. More studies are necessary to clarify the taxonomy and species diversity of the genus *Brachytarsophrys* in Guangxi, China.

Key Words

Acoustic, phylogenetic analysis, species diversity, taxonomy

Introduction

The genus *Brachytarsophrys* Tian & Hu, 1983, commonly known as short-legged toads, is characterized by large body size; thickset and stout habitus; head enormous and extremely depressed, with head width approximately twice the skull length; presence of a transverse groove defining the head behind; tympanum hidden; maxillary teeth present; pupil vertical; upper eyelid bearing several conical tubercles, one elongated, forming a conical or flattened horn; hindlimbs short and strongly thickset, heels not meeting and separated by a greater distance; toes with webbing and fringes (Li et al. 2020). The genus is widely distributed in southern China, Myanmar, Vietnam, Laos, and northern Thailand, and currently comprises nine species (Frost 2025). In China, seven species have been recorded: *B. chuannanensis* Fei, Ye & Huang, 2001; *B. feae* (Boulenger, 1887); *B. orientalis* Li, Lyu, Wang & Wang, 2020; *B. platyparietus* Rao & Yang, 1997; *B. popei* Zhao, Yang, Chen, Chen & Wang, 2014; *B. qiannanensis* Li, Liu, Yang, Wei & Su, 2022; and *B. wenshanensis* He, Huang, Li, Chen & Yuan, 2024 (AmphibiaChina 2025). Of these seven species, only *B. platyparietus* is recognized in Guangxi (Li et al. 2020; AmphibiaChina 2025).

During field surveys conducted in September 2024 in Guangxi, China, we collected eight specimens of *Brachytarsophrys*. Both morphological and phylogenetic analyses confirmed that these specimens represent an unde-

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scribed species of *Brachytarsophrys*, suggesting that the species diversity within the genus *Brachytarsophrys* has been underestimated.

Materials and methods

Sampling

This study was carried out in accordance with the ethical guidelines issued by the Ethics Committee of Guangxi Normal University. A total of eight specimens of the genus *Brachytarsophrys* were collected in September 2024 from Wantian Township, Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, China (Fig. 1). All specimens were photographed, euthanized, fixed, and stored in 75% ethanol. Liver tissues were preserved in 99% ethanol. All specimens were deposited at Guangxi Normal University (GXNU).

Morphological analysis

Morphometric data were taken using electronic digital calipers to the nearest 0.1 mm. Morphological terminology followed Fei (1999). Measurements included: snout–vent length (SVL, from tip of snout to vent); head length (HL, from tip of snout to rear of jaws); head width (HW, width of head at its widest point); snout length (SL, from tip of snout to anterior corner of eye); internarial distance (IND, distance between nares); interorbital distance (IOD, minimum distance between upper eyelids); eye diameter (ED, diameter of exposed portion of eyeball); hand length (HAL, from outer palm to the end of third finger); forearm length (FOL, from proximal end of outer palm protrusion to elbow joint); tibia length (TL, distance from knee to heel); and foot length (FL, from proximal end of inner metatarsal tubercle to tip of fourth toe). The webbing formula followed Myers and Duellman (1982).

Comparative morphological data of known *Brachytarsophrys* species were taken from their original and subsequent descriptions (Boulenger 1887, 1889; Smith 1921; Taylor 1962; Rao and Yang 1997; Fei and Ye 2001; Fei et al. 2009; Zhao et al. 2014; Li et al. 2020, 2022; He et al. 2024).

Multivariate principal component analysis (PCA) was conducted in SPSS v.25.0 (SPSS Inc.) based on the correlation matrix of size-standardized measurements (all measurements divided by SVL) of adult males. Scatter plots of the scores of the first two principal components were used to examine differentiation between the new species and its closest relatives as revealed by phylogenetic analyses (*Brachytarsophrys popei* only; data for *B. orientalis* were not available). Differences in quantitative characters of adult males between these two species were also evalu-

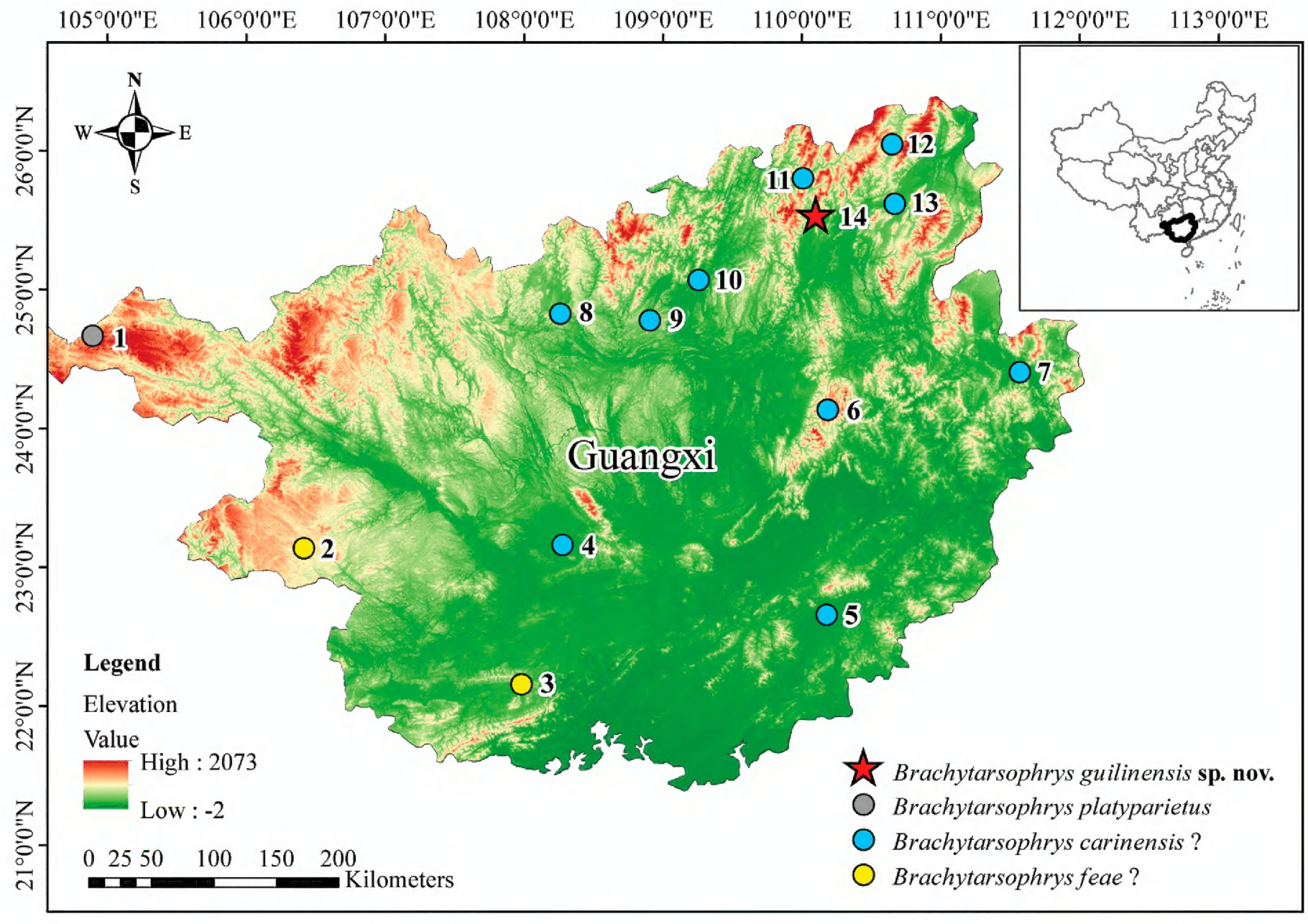

Figure 1. Map showing the distribution of the *Brachytarsophrys* species in Guangxi. The red star indicates the type locality of *Brachytarsophrys guilinensis* sp. nov. The "?" in the legend indicates that the species identity is yet to be confirmed. 1: Longlin; 2: Jingxi; 3: Shangsi; 4: Wuming; 5: Yulin; 6: Jinxiu; 7: Hezhou; 8: Huanjiang; 9: Luocheng; 10: Rongshui; 11: Longsheng; 12: Ziyuan; 13: Xing'an (Fei et al. 2009, 2012; Mo et al. 2014). The map was produced using ArcMap v.10.8.1.

ated with t-tests in SPSS. For each character, Levene's test was performed to assess equality of variances.

Acoustic analysis

Acoustic data were recorded in the field using a Sony PCM-A10 recorder. Ambient temperature and humidity were measured using a digital hygrothermograph (DELIXI). Recordings were resampled at 44.1 kHz and 16-bit resolution and analyzed using Adobe Audition (Adobe Inc.). The software Praat v.6.2.14 (Boersma 2001) was used to obtain waveforms and sonograms. Terminology and description of advertisement call analyses followed Köhler et al. (2017).

Phylogenetic analysis

Total genomic DNA was extracted from liver tissue preserved in 99% ethanol. Three mitochondrial genes – 16S ribosomal RNA (16S rRNA), cytochrome oxidase subunit I (COI), and cytochrome b (Cytb) – were amplified and sequenced. For 16S rRNA, primers L2188 and 16H1 were used following Matsui and Panha (2006) and Hedges (1994), respectively; for COI, primers Chmf4 and Chmr4 were used following Che et al. (2012); and for Cytb, primers PFGlu14140L and PFThr15310 were used following Zhang et al. (2013). Gene fragments were amplified in a 25 μL volume with the following PCR conditions: initial denaturation at 95 °C for 4 min; 35 cycles of denaturation at 95 °C for 30 s, annealing at 49 °C–54 °C (54 °C for 16S rRNA, 49 °C for COI, and 50 °C for Cytb) for 40 s, and extension at 72 °C for 70 s. PCR products were sequenced using an ABI PRISM 3730 automatic sequencer. New sequences have been deposited in GenBank (Table 1). A total of 105 homologous sequences from all known *Brachytarsophrys* species, as well as an outgroup, were downloaded from GenBank (Table 1). *Atympanophrys shapingensis* (Liu, 1950) was included as the outgroup.

Sequences were aligned using MUSCLE in MEGA v.7.0 (Kumar et al. 2016) with default parameters. Uncorrected pairwise distances between *Brachytarsophrys* species were calculated for each locus in MEGA v.7.0. The best nucleotide substitution model for the combined dataset was selected using the Akaike Information Criterion (AIC) in PartitionFinder v.2.1.1 (Robert et al. 2017), with GTR+G+I identified as the best-fit model for all three genes. Bayesian inference was performed using the combined data in MrBayes v.3.2.6 (Ronquist et al. 2012) under the selected model. Two runs were performed, each with four Markov chains starting from a random tree. Chains were run for 5,000,000 generations and sampled every 1,000 generations. The first 25% of sampled trees were discarded as burn-in after the standard deviation of split frequencies between runs dropped below 0.01. The remaining trees were used to generate a consensus tree and to estimate Bayesian posterior probabilities (BPPs). Maximum likelihood phylogenetic analysis was conducted in IQ-TREE v.2.2.0 (Nguyen et al. 2015), with nodal support estimated using 5,000 ultrafast bootstrap replicates. Species delimitation analysis was performed using the p-distance model based on COI sequences on the ASAP webserver (https://bioinfo.mnhn.fr/abi/public/asap/) (Puillandre et al. 2021).

Results

Morphological study

Morphological data were summarized in Table 2. Two measurements (UEW and FHL) were not included in PCA because they were not obtainable for *Brachytarsophrys popei*. The t-tests revealed that male specimens from Guilin significantly ($p < 0.05$) differed from male specimens of *B. popei* from Jiangxi, Guangdong, and Yanling, Hunan, in HL, HW, SL, IOD, ED, FOL, and FL (Table 3). We retained the first two principal components that accounted for 71.794% of the total variance (Table 4). Loadings for PC1, which account for 51.580% of the total variance, were most heavily loaded on HL, HW, SL, ED, FOL, and FL. Loadings for PC2, which account for 20.214% of the total variance, were most heavily loaded on IND and TL. Differentiation was found along the PC1 axis between the specimens from Guilin and *B. popei* (Fig. 2). This result indicates that specimens from Guilin differ from *B. popei* in several characteristics, such as shorter and narrower head, smaller eye diameter, shorter snout length, forearm length, and foot length. Along the PC2 axis, no differentiation was observed between the specimens from Guilin and *B. popei*.

Phylogenetic relationship

The obtained sequence alignments for 16S rRNA, COI, and Cytb genes resulted in a combined dataset of 2181 bp in length. Phylogenetic analyses revealed that the specimens from Guilin, Guangxi, form a distinct clade with strong support (100% and 100% for BI and ML, respectively), and this clade was closer to *Brachytarsophrys popei* and *B. orientalis* than to other known species, with strong Bayesian posterior probability and moderate bootstrap support (100% and 88% for BI and ML, respectively) (Fig. 3). Genetic distances (uncorrected p-distances) between this novel lineage and other *Brachytarsophrys* species ranged from 2.1% to 8.9% in 16S rRNA sequences (Suppl. material 1), from 3.5% to 15.8% in COI sequences (Suppl. material 2), and from 3.7% to 15.5% in Cytb sequences (Suppl. material 3). The results of species delimitation obtained by the ASAP analysis indicated that the specimens from Guilin represent an operational taxonomic unit within the genus *Brachytarsophrys* (Fig. 4). In addition, it was revealed that *B. popei* is composed of two distinct lineages, one containing samples from the type locality (Yanling, eastern Hunan) and Jinggang Mountain, western Jiangxi, and one containing samples from Mangshan (southern Hunan) and adjacent Nanling Nature Reserve, Guangdong (Fig. 3). Furthermore, the analysis of ASAP species delimitation assigned these two lineages to two operational taxonomic units (Fig. 4).

Table 1. Species used for phylogenetic analyses in this study (*B.* = *Brachytarsophrys*).

Species	Locality	Voucher No.	16S	COI	Cytb	References
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000903	PV819239	PV817819	PV820718	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000904	PV819240	PV817820	PV820719	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000905	PV819241	PV817821	PV820720	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000906	PV819242	PV817822	PV820721	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000907	PV819243	PV817823	PV820722	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000908	PV819244	PV817824	PV820723	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000909	PV819245	PV817825	PV820724	This study
B. guilinensis sp. nov.	Guilin, Guangxi, China	GXNU YU000910	PV819246	PV817826	PV820725	This study
B. orientalis	Longnan County, Jiangxi, China	SYS a004225	OQ180989	MT162625	MT162650	Li et al. (2020); Lyu et al. (2023)
B. orientalis	Longnan County, Jiangxi, China	SYS a004226	–	MT162626	MT162651	Li et al. (2020)
B. orientalis	Longnan County, Jiangxi, China	SYS a004227	–	MT162627	MT162652	Li et al. (2020)
B. orientalis	Longnan County, Jiangxi, China	SYS a004228	–	MT162628	MT162653	Li et al. (2020)
B. orientalis	Longnan County, Jiangxi, China	SYS a004486	–	MT162629	MT162654	Li et al. (2020)
B. orientalis	Longnan County, Jiangxi, China	SYS a005451	–	MT162632	MT162655	Li et al. (2020)
B. orientalis	Shanghang County, Fujian, China	SYS a003249	–	MT162623	MT162648	Li et al. (2020)
B. orientalis	Nanjing County, Fujian, China	SYS a003340	–	MT162624	MT162649	Li et al. (2020)
B. carinense	Doi Chiang Dao, Chiang Mai, Thailand	K3001	KR827713	KR087626	–	Grosjean et al. (2015)
B. carinense	Omkoi, Chiang Mai, Thailand	KIZ 024170	–	MT162640	MT162663	Li et al. (2020)
B. carinense	Mae Surin NP., Mae Hong Son, Thailand	KIZ 024429	–	MT162641	MT162664	Li et al. (2020)
B. carinense	Thong Pha Phum, Kanchanaburi, Thailand	KIZ 024640	–	MT162642	MT162665	Li et al. (2020)
B. chuannanensis	Hejiang County, Sichuan, China	SYS a004926	MH406901	MH406364	MH407194	Liu et al. (2018)
B. chuannanensis	Hejiang County, Sichuan, China	SYS a004927	MH406902	MH406365	MH407195	Liu et al. (2018)
B. feae	Jingdong County, Yunnan, China	SYS a003912	MH406899	MH406362	MH407192	Liu et al. (2018)
B. feae	Jingdong County, Yunnan, China	SYS a003913	MH406900	MH406363	MH407193	Liu et al. (2018)
B. feae	Huangcaoling, Yunnan, China	KIZ 046706	KX811810	KX812056	–	Chen et al. (2017)
B. intermedia	Krong Pa, Gia Lai, Vietnam	ROM 23794	–	MT162643	MT162666	Li et al. (2020)
B. platyparietus	Dayao county, Yunnan, China	SYS a005919	OQ180990	MT162633	MT162656	Li et al. (2020); Lyu et al. (2023)
B. platyparietus	Longlin County, Guangxi, China	SYS a002236	–	MT162622	MT162647	Li et al. (2020)
B. platyparietus	Tongren City, Guizhou, China	YPX43968	–	MT162644	MT162667	Li et al. (2020)
B. platyparietus	Xinping County, Yunnan, China	SYS a007774	–	MT162634	MT162657	Li et al. (2020)
B. platyparietus	Xinping County, Yunnan, China	SYS a007775	–	MT162635	MT162658	Li et al. (2020)
B. platyparietus	Xinping County, Yunnan, China	SYS a007776	–	MT162636	MT162659	Li et al. (2020)
B. platyparietus	Xinping County, Yunnan, China	SYS a007777	–	MT162637	MT162660	Li et al. (2020)
B. platyparietus	Shiping County, Yunnan, China	SYS a007790	–	MT162638	MT162661	Li et al. (2020)
B. platyparietus	Yanbian County, Sichuan, China	SYS a007853	–	MT162639	MT162662	Li et al. (2020)
B. popei	Yanling County, Hunan, China	SYS a001864	KM504256	MH406361	MH407191	Zhao et al. (2014); Liu et al. (2018)
B. popei	Yanling County, Hunan, China	SYS a001865	KM504257	MT162620	MT162645	Zhao et al. (2014); Li et al. (2020)
B. popei	Yanling County, Hunan, China	SYS a001866	KM504258	MT162621	MT162646	Zhao et al. (2014); Li et al. (2020)

Species	Locality	Voucher No.	16S	COI	Cytb	References
B. popei	Mt.Jinggang, Jiangxi, China	SYS a001876	KM504253	–	–	Zhao et al. (2014)
B. popei	Mt.Jinggang, Jiangxi, China	SYS a001877	KM504254	–	–	Zhao et al. (2014)
B. popei	Mt.Jinggang, Jiangxi, China	SYS a001878	KM504255	–	–	Zhao et al. (2014)
B. popei	Mt.Jinggang, Jiangxi, China	SYS a004209	MK524124	MK524155	–	Wang et al. (2019)
B. popei	Mt.Jinggang, Jiangxi, China	SYS a001485-1	KM504252	–	–	Zhao et al. (2014)
B. popei	Tiantaishan, Mangshan, Hunan, China	CSUFT T10115	ON209276	–	–	–
B. popei	Tiantaishan, Mangshan, Hunan, China	CSUFT T10117	ON209284	–	–	–
B. popei	Nanling Nature Reserve, Guangdong, China	SYS a000589	KM504251	–	–	Zhao et al. (2014)
B. popei	Nanling Nature Reserve, Guangdong, China	YTP34994	PV819247	PV819270	PV820726	This study
B. popei	Nanling Nature Reserve, Guangdong, China	YTP34995	PV819248	PV819271	PV820727	This study
B. popei	Nanling Nature Reserve, Guangdong, China	YTP34996	PV819249	PV819272	PV820728	This study
B. qiannanensis	Libo County, Guizhou, China	CIB LB20210806053	OK104099	OK104052	OK127913	Li et al. (2022)
B. qiannanensis	Libo County, Guizhou, China	CIB LB20210806054	OK104100	OK104053	OK127914	Li et al. (2022)
B. qiannanensis	Libo County, Guizhou, China	CIB LB20210806055	OK104101	OK104054	OK127915	Li et al. (2022)
B. qiannanensis	LiboCounty, Guizhou, China	CIB LB20210806056	OK104102	OK104055	OK127916	Li et al. (2022)
B. wenshanensis	Xichou, Yunnan, China	SWU 0004072	PP153337	PP155195	PP156671	He et al. (2024)
B. wenshanensis	Xichou, Yunnan, China	SWU 0002976	PP153338	PP155198	PP156670	He et al. (2024)
B. wenshanensis	Xichou, Yunnan, China	Yuan 25290	PP153339	PP155197	PP156669	He et al. (2024)
B. wenshanensis	Xichou, Yunnan, China	Yuan 25291	PP153336	PP155196	PP156668	He et al. (2024)
Atympanophrys shapingensis	Hongya County, Sichuan, China	SYS a005310	MH406890	MH406352	MH407182	Liu et al. (2018)

–: Not available.

Table 2. Measurements (mm) of *Brachytarsophrys guilinensis* sp. nov. (1–8) and *B. popei* (9–21). Data of *B. popei* were obtained from Zhao et al. (2014).

ID	Voucher No.	Sex	SVL	HL	HW	SL	IND	IOD	ED	HAL	FOL	TL	FL
1	GXNU YU000903	M	77.3	25.3	36.9	8.7	8.4	11.1	8.2	19.7	15.4	32.0	34.1
2	GXNU YU000904	M	74.6	22.6	33.3	8.4	7.4	11.0	7.1	18.7	14.3	28.0	30.4
3	GXNU YU000905	M	76.2	26.2	39.0	8.6	8.2	10.9	8.3	19.9	15.7	31.9	32.7
4	GXNU YU000906	M	76.4	25.7	37.9	8.9	7.7	11.7	8.4	19.4	15.6	31.7	32.4
5	GXNU YU000907	M	73.7	22.7	33.8	8.4	7.3	10.7	7.1	19.1	14.2	28.5	30.5
6	GXNU YU000908	M	71.0	21.6	33.5	8.4	7.4	10.4	6.6	19.1	13.9	28.7	30.6
7	GXNU YU000909	M	70.0	21.5	31.8	8.1	7.3	10.3	6.6	18.9	13.7	27.9	30.6
8	GXNU YU000910	M	81.9	27.8	40.4	9.8	8.3	11.4	8.6	21.4	16.5	32.4	35.4
9	SYS a001874	M	79.8	33.2	40.1	10.3	7.4	12.0	11.1	20.8	17.5	32.2	47.4
10	SYS a001876	M	75.1	32.3	38.6	9.4	7.8	11.2	9.6	19.3	17.4	32.2	46.3
11	SYS a001877	M	83.5	33.4	39.9	10.0	8.4	14.0	10.6	20.6	17.0	32.4	48.4
12	SYS a001878	M	75.8	32.9	39.5	9.9	7.9	11.6	9.2	20.6	15.6	30.4	45.3
13	SYS a001864	M	78.4	32.7	40.8	10.2	8.0	11.9	9.9	21.6	17.1	32.2	49.5
14	SYS a001865	M	78.4	31.0	37.2	9.7	7.9	11.2	8.9	19.4	17.0	30.4	45.0
15	SYS a001866	M	79.5	34.2	41.6	9.6	8.7	13.4	10.0	20.5	17.5	33.5	49.7
16	SYS a001867	M	72.9	30.8	36.3	8.9	7.5	12.2	7.8	19.1	16.1	28.9	43.9
17	SYS a000583	M	70.7	29.8	36.9	8.5	7.0	11.1	7.9	18.6	15.7	28.0	42.0
18	SYS a000584	M	72.6	30.6	38.7	9.4	7.7	12.3	8.0	18.8	15.9	29.4	45.8
19	SYS a000585	M	79.3	33.2	40.0	10.2	7.7	11.8	9.7	20.2	17.9	30.1	46.6
20	SYS a000588	M	76.5	32.3	40.0	9.8	7.6	11.7	8.4	19.3	18.0	30.8	46.2
21	SYS a000589	M	76.8	32.5	38.4	9.2	7.4	11.1	9.3	18.8	16.8	29.4	43.8

Table 3. Summary statistics of male specimens (mean ± standard deviation) and results of the t-test between *Brachytarsophrys guilinensis* sp. nov. (n = 8) and *B. popei* (n = 13). The t-test was performed on the size-adjusted data, except for SVL. * = $p < 0.05$.

Character	Mean ± SD (n = 8)	Mean ± SD (n = 13)	Levene's test		t-test	
	***B. guilinensis* sp. nov.**	***B. popei***	**F**	***p*-value**	**t**	***p*-value**
SVL	75.1 ± 3.7	76.9 ± 3.5	0.010	0.920	-1.069	0.299
HL	0.321 ± 0.0175	0.419 ± 0.0110	7.039	0.016	-14.276	0.000*
HW	0.476 ± 0.0232	0.509 ± 0.0180	0.720	0.407	-3.614	0.002*
SL	0.115 ± 0.0025	0.125 ± 0.0043	7.102	0.015	-6.658	0.000*
IND	0.103 ± 0.0038	0.101 ± 0.0044	0.023	0.881	1.112	0.280
IOD	0.146 ± 0.0041	0.156 ± 0.0095	7.807	0.012	-3.362	0.004*
ED	0.101 ± 0.0072	0.120 ± 0.0093	0.253	0.621	-4.994	0.000*
HAL	0.260 ± 0.0067	0.258 ± 0.0091	0.561	0.463	0.547	0.591
FOL	0.198 ± 0.0054	0.220 ± 0.0086	0.244	0.627	-6.359	0.000*
TL	0.401 ± 0.0150	0.400 ± 0.0144	0.067	0.798	0.099	0.922
FL	0.427 ± 0.0112	0.601 ± 0.0205	2.986	0.100	-21.874	0.000*

Table 4. Factor loadings of first two principal components of 10 size-adjusted male morphometric characteristics of *Brachytarsophrys guilinensis* sp. nov. and *B. popei*. Absolute values of loading greater than 0.75 in boldface. Abbreviations are defined in Materials and methods.

Character	PC1	PC2
Eigenvalue	5.158	2.021
% variation	51.580%	20.214%
HL	**0.973**	-0.114
HW	**0.853**	0.310
SL	**0.853**	-0.119
IND	-0.108	**0.875**
IOD	0.554	0.192
ED	**0.811**	-0.146
HAL	0.022	0.619
FOL	**0.886**	-0.117
TL	0.275	**0.819**
FL	**0.959**	-0.084

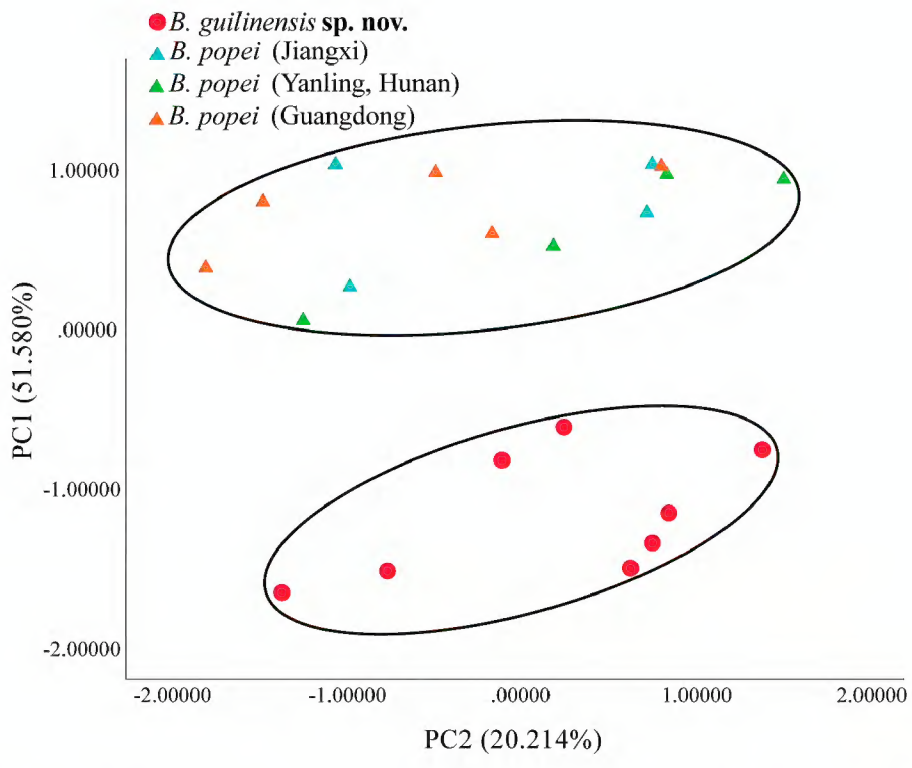

Figure 2. Scatterplot of principal components 1 and 2 of size-adjusted male morphometric data of *Brachytarsophrys guilinensis* sp. nov. and *B. popei*.

Based on the combination of morphological and phylogenetic evidence mentioned above, we consider that the individuals from Wantian Township, Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, China, represent a new species of the genus *Brachytarsophrys*, which we describe herein.

Taxonomic account

Brachytarsophrys guilinensis sp. nov.

https://zoobank.org/8AE13F98-2D19-4B78-A9F8-A4BB1DE906E5

Figs 5–8, 11

Type material. ***Holotype*** • GXNU YU000904, an adult male (Fig. 5), collected on 6 September 2024 by Guohua Yu, Yuanqiang Pan, and Wei Xiao from Wantian Township, Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, China (25°31'53.54"N, 110°2'43.12"E, 289 m a.s.l; Fig. 1).

Paratypes • GXNU YU000903 and YU000905–YU000910, seven adult males, collected on 6 September 2024 by Guohua Yu, Yuanqiang Pan, and Wei Xiao from the type locality.

Etymology. The species name *guilinensis* is derived from the type locality of this species, Guilin City, Guangxi Zhuang Autonomous Region, China. We suggest the English common name "Guilin Short-legged Toad" and the Chinese common name "Guì Lín Duǎn Tuǐ Chán (桂林短腿蟾)".

Diagnosis. *Brachytarsophrys guilinensis* sp. nov. could be distinguished from its congeners by the combination of the following morphological characters: (1) smaller body size (SVL 70.0–81.9 mm in eight adult males); (2) head wider than long, head width nearly 1.5 times the length, and about half of the SVL; (3) tongue broad and round, feebly notched; (4) male with nuptial pad present on the dorsal surface of the first and second fingers; (5) hind limbs relatively short and robust, heels do not meet when legs positioned at right angle to body; (6) tibiotarsal artic-

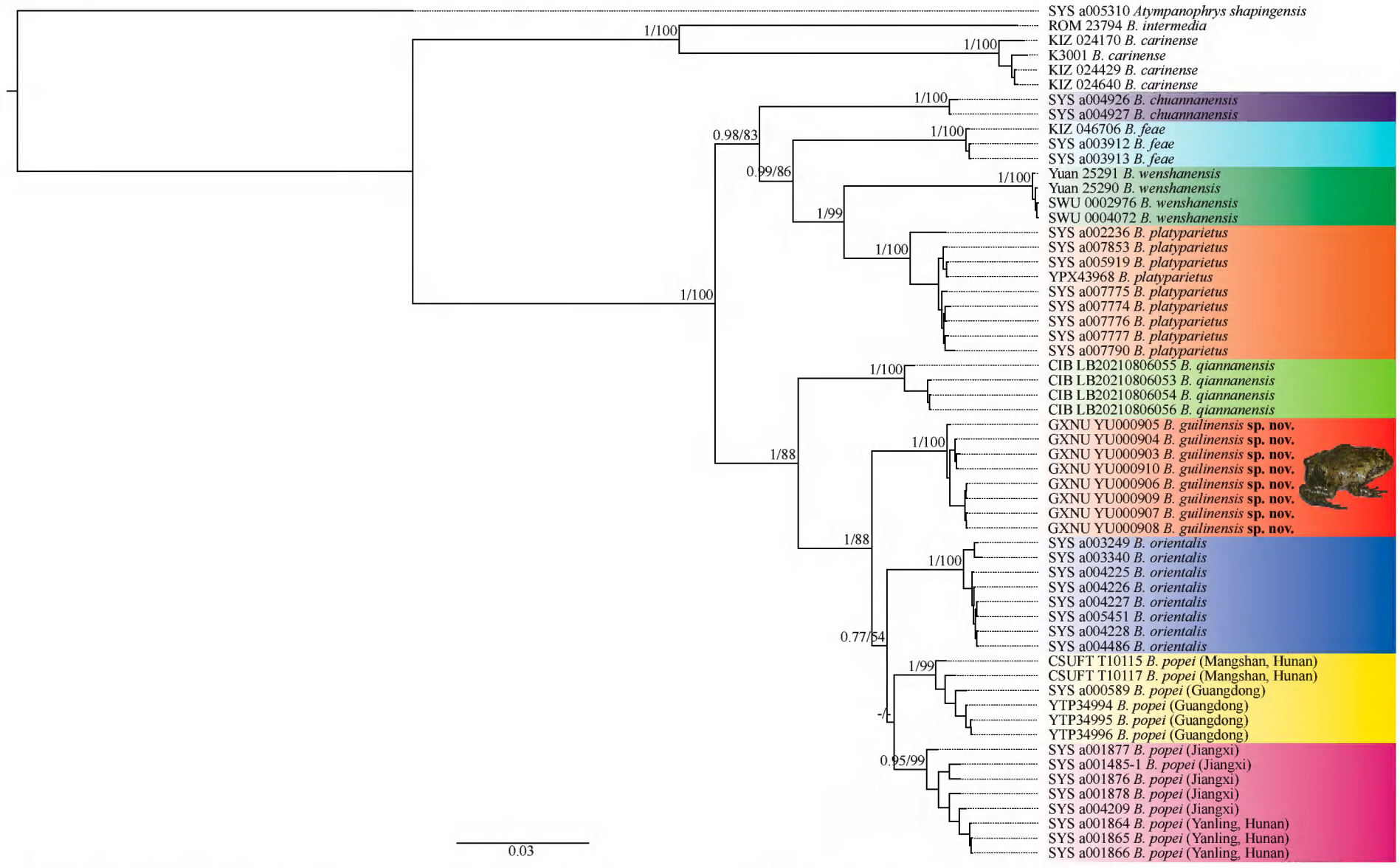

Figure 3. Bayesian phylogram of *Brachytarsophrys* inferred from 16S rRNA, COI, and Cytb genes. Numbers above and below branches are Bayesian posterior probabilities and ML bootstrap values (only values above 50% are shown), respectively.

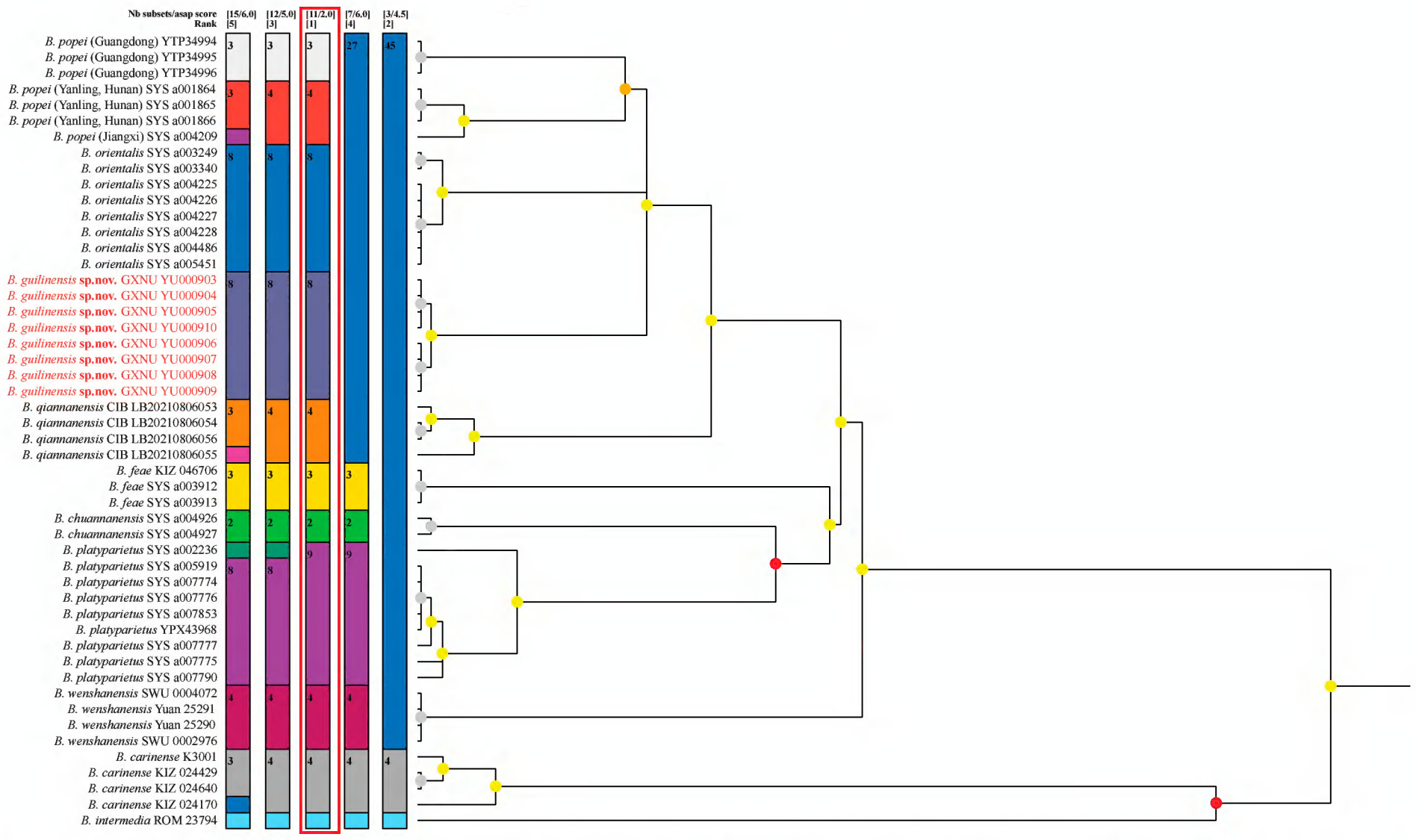
Nb subsets/asap score
Rank
[15/6.0] [5]
[12/5.0] [3]
[11/2.0] [1]
[7/6.0] [4]
[3/4.5] [2]
B. popei (Guangdong) YTP34994
B. popei (Guangdong) YTP34995
B. popei (Guangdong) YTP34996
B. popei (Yanling, Hunan) SYS a001864
B. popei (Yanling, Hunan) SYS a001865
B. popei (Yanling, Hunan) SYS a001866
B. popei (Jiangxi) SYS a004209
B. orientalis SYS a003249
B. orientalis SYS a003340
B. orientalis SYS a004225
B. orientalis SYS a004226
B. orientalis SYS a004227
B. orientalis SYS a004228
B. orientalis SYS a004486
B. orientalis SYS a005451
B. guilinensis sp.nov. GXNU YU000903
B. guilinensis sp.nov. GXNU YU000904
B. guilinensis sp.nov. GXNU YU000905
B. guilinensis sp.nov. GXNU YU000910
B. guilinensis sp.nov. GXNU YU000906
B. guilinensis sp.nov. GXNU YU000907
B. guilinensis sp.nov. GXNU YU000908
B. guilinensis sp.nov. GXNU YU000909
B. qiannanensis CIB LB20210806053
B. qiannanensis CIB LB20210806054
B. qiannanensis CIB LB20210806056
B. qiannanensis CIB LB20210806055
B. feae KIZ 046706
B. feae SYS a003912
B. feae SYS a003913
B. chuannanensis SYS a004926
B. chuannanensis SYS a004927
B. platyparietus SYS a002236
B. platyparietus SYS a005919
B. platyparietus SYS a007774
B. platyparietus SYS a007776
B. platyparietus SYS a007853
B. platyparietus YPX43968
B. platyparietus SYS a007777
B. platyparietus SYS a007775
B. platyparietus SYS a007790
B. wenshanensis SWU 0004072
B. wenshanensis Yuan 25291
B. wenshanensis Yuan 25290
B. wenshanensis SWU 0002976
B. carinense K3001
B. carinense KIZ 024429
B. carinense KIZ 024640
B. carinense KIZ 024170
B. intermedia ROM 23794

Figure 4. Species delimitation results obtained by ASAP based on COI sequences. Rank 1 represents the best partition, identifying 11 species within the *Brachytarsophrys* genus.

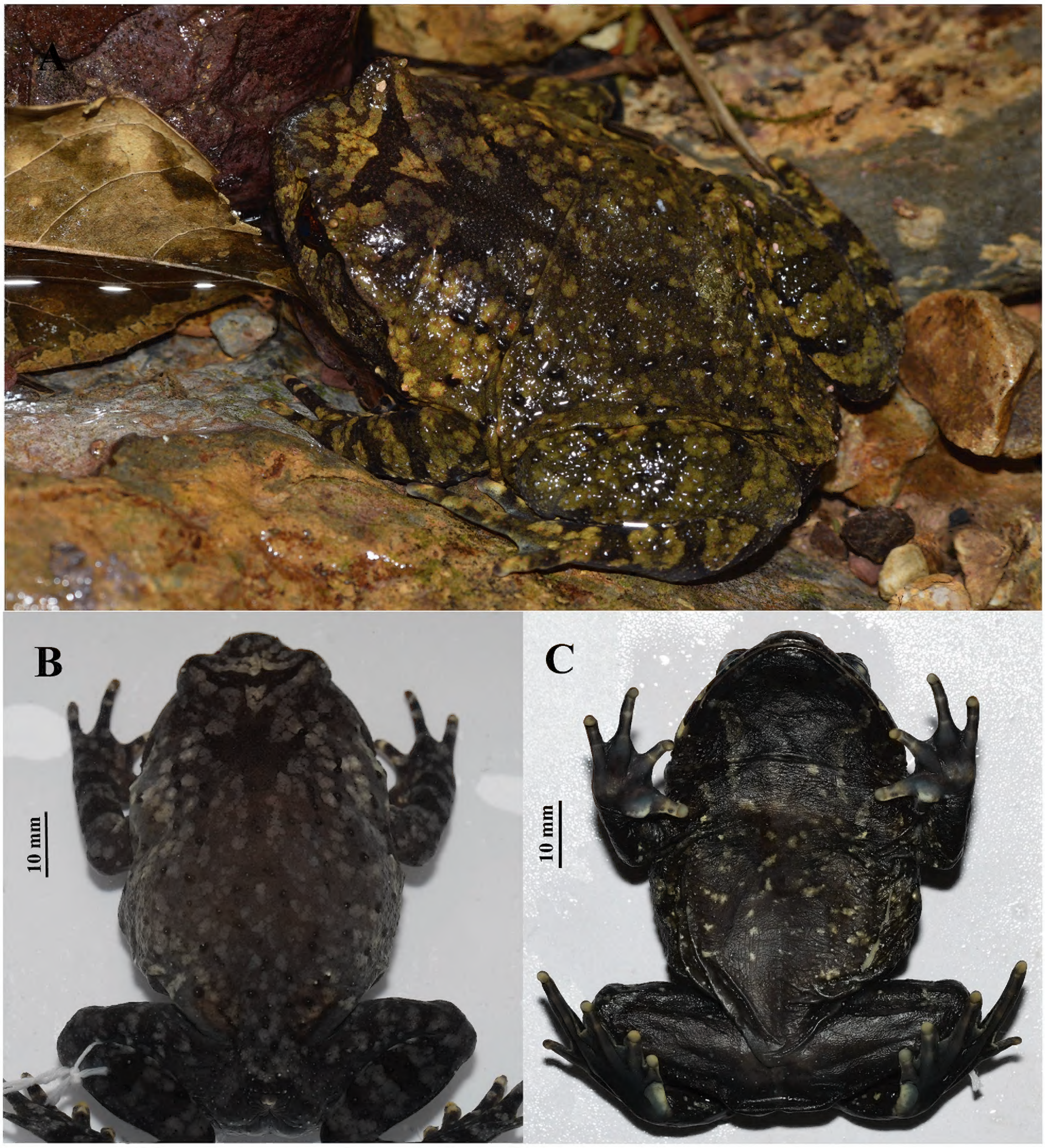

Figure 5. Dorsolateral view of the holotype of *Brachytarsophrys guilinensis* sp. nov. (GXNU YU000904) in life (**A**) and dorsal (**B**) and ventral (**C**) views of the holotype of the species in preservative.

ulation reaches the angle of mouth when the hind limbs are extended forward alongside the body; (7) absence of outer metatarsal tubercle, inner metatarsal tubercle oval and approximately equal to first toe; (8) fingers without webbing, toes have webbing but incomplete, webbing formula: I1 - 2II1 - 2III1½ - 3-IV3- - 1½V; (9) lateral fringes on toes wide; (10) several warts on the outer side of the upper eyelid, with one being relatively larger.

Description of holotype. Adult male; body robust, SVL 74.6 mm; head enormous, extremely wide and flat, about 1.5 times as broad as long, and nearly one-half as broad as the SVL; snout short, rounded in dorsal view, slightly protruding beyond margin of lower jaw in ventral view, sloping in profile; canthus rostralis distinct; loreal region sloping, slightly concave; nostrils oval, much closer to eye than to snout tip; interorbital distance (11.0 mm) significantly wider than the width of upper eyelid, and wider than internasal distance (7.4 mm); occipital region slightly uplifted, forming two oval bulges and a longitudinal concave groove along the middle line across occiput; a distinct transverse groove behind the head; pupil oval, vertical; tympanum hidden; choanae large, circu-

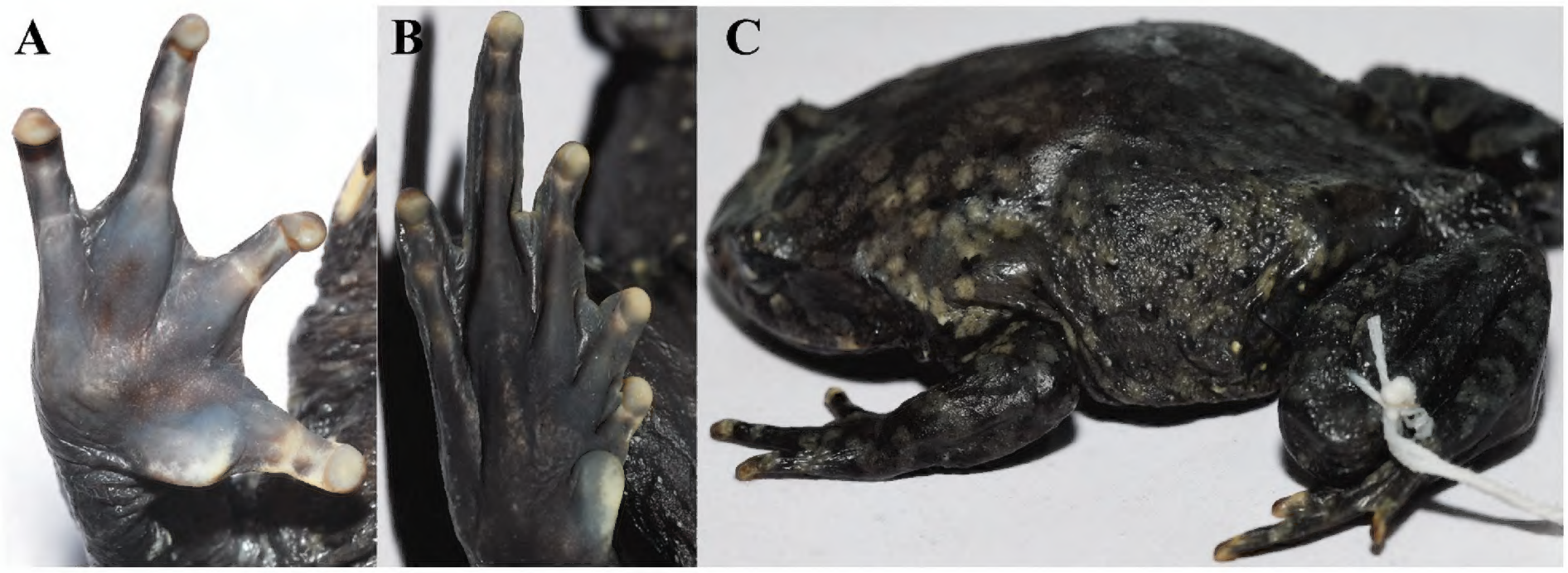

Figure 6. Ventral view of hand (**A**) and foot (**B**) and lateral view (**C**) of the holotype of *Brachytarsophrys guilinensis* sp. nov. (GXNU YU000904) in preservative.

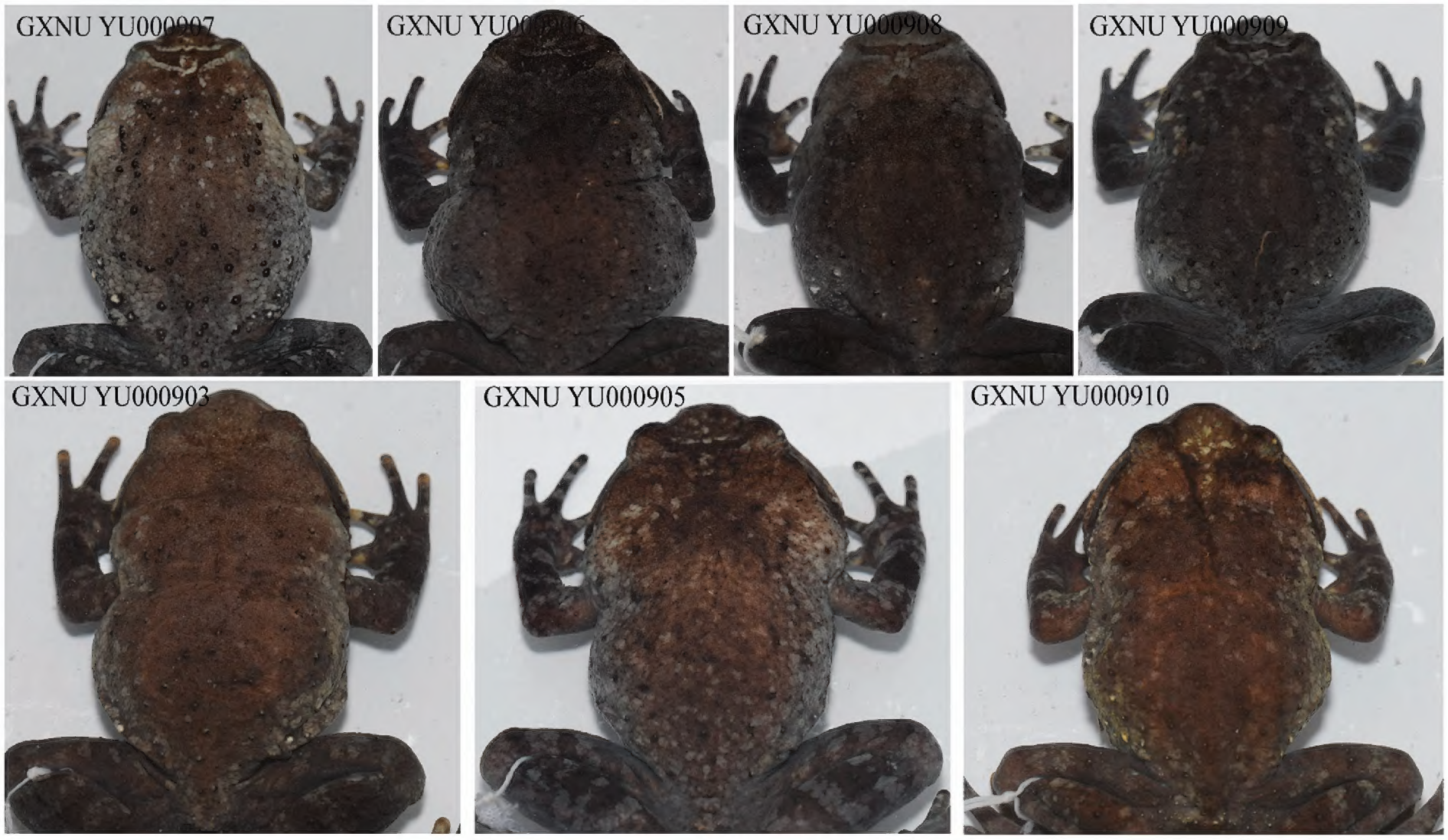

Figure 7. Variation in dorsal skin coloration of *Brachytarsophrys guilinensis* sp. nov. in preservative.

lar, partly concealed by the maxillary shelves; vomerine teeth present in two series, touching inner front edges of choanae, separated by distance larger than length of each series; tongue broad and round, feebly notched posteriorly; an internal single subgular vocal sac with a vocal sac opening at each corner of mouth.

Forelimbs short and robust; relative length of fingers I = II < IV < III; tips of fingers round, feebly dilated; no lateral fringes and webbing between fingers; nuptial pad on dorsal surface of the first and second fingers; inner metacarpal tubercle large, oval; outer metacarpal tubercle inconspicuous (Fig. 6A).

Hindlimbs relatively short and robust; heels do not meet when legs positioned at right angle to body; tibiotarsal articulation reaching angle of mouth when hind limbs are extended forward alongside the body; tips of toes round, moderately dilated; relative length of toes I < II < V < III < IV; toes have webbing but incomplete, webbing formula: I1 - 2II1 - 2III1½ - 3-IV3- - 1½V; lateral fringes on toes wide; subarticular tubercle absent; inner metatarsal tubercle distinct, oval, approximately equal to first toe; outer metatarsal tubercle absent (Fig. 6B).

Dorsal and lateral skin rough, covered with some slightly larger black glandular warts and small granules (Fig. 6C); several warts on the outer side of the upper eyelid, with one being relatively larger; ventral skin smooth, with some white granules scattered on abdomen and ventral sides of the limbs; pectoral glands distinct, light yellow, with one on each side near axilla; femoral glands smaller than pectoral glands, white, with one on each side of rear of thigh.

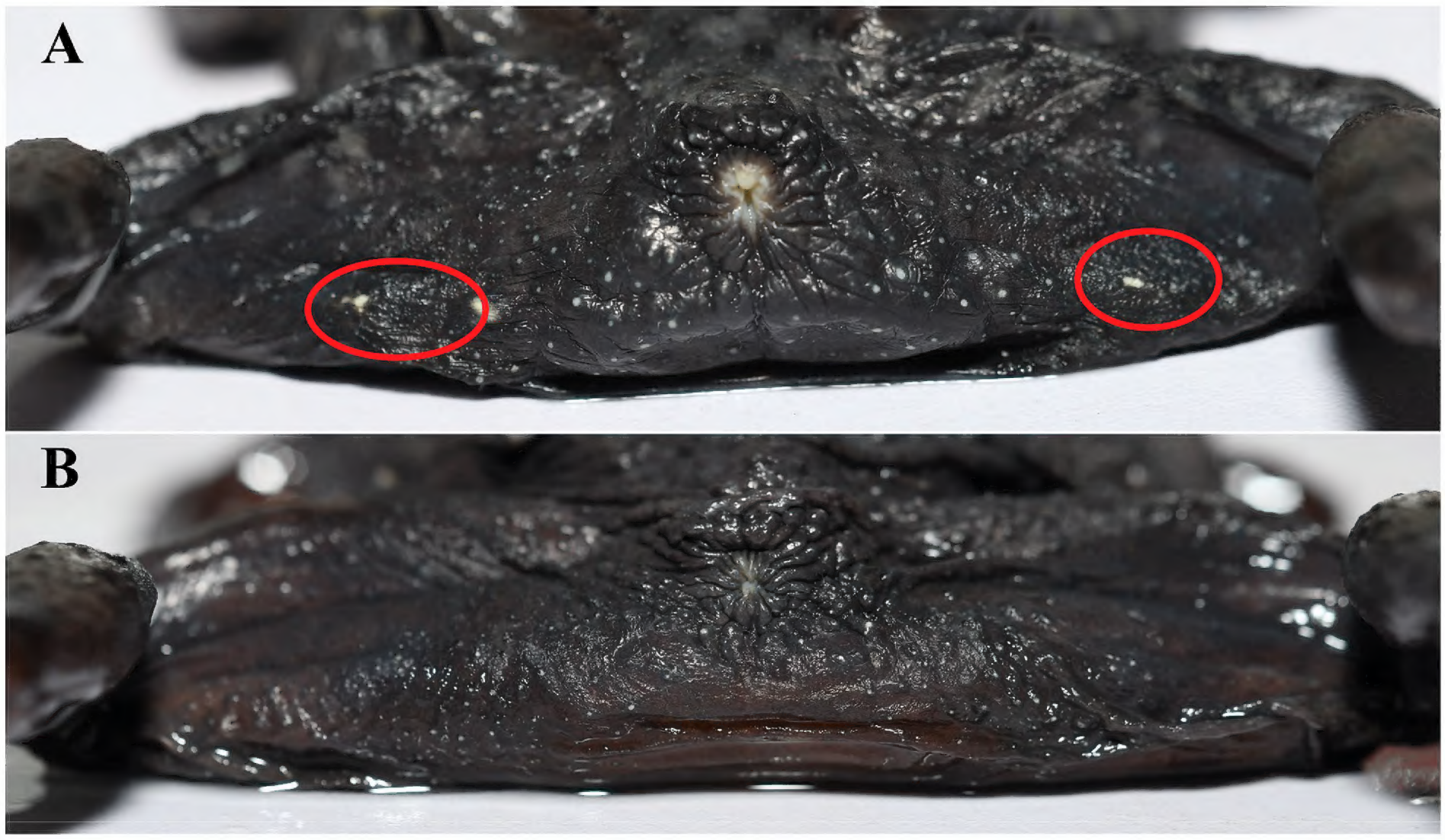

Figure 8. Difference in the femoral gland between holotype (GXNU YU000904, **A**) and paratype (GXNU YU000906, **B**). The femoral gland is indicated by the red circle.

Coloration of holotype in life. Iris red; dorsal surface yellow-brown, scattered with yellow patches (Fig. 5A); black stripe between eyes, patch to back of stripe black; tympanic region brown; dorsal glandular warts black; limbs dorsal sides with multiple black stripes; ventral surface dark brown, scattered with yellow patches and white granules; ventral sides of limbs with white granules; pectoral gland light yellow; femoral gland white.

Coloration of holotype in preservative. Dorsal skin dark grey, scattered with grey-white patches; interocular stripe black, patch to back of stripe black; limbs dorsal stripes black (Fig. 5B); ventral patches grey-white; abdomen and limbs ventral granules white; pectoral gland white (Fig. 5C); femoral gland white.

Sexual dimorphism. Adult males have a nuptial pad on the dorsal surface of the first and second fingers. Additionally, males have an internal single subgular vocal sac with a vocal sac opening at each corner of the mouth.

Variation. Coloration of three paratypes (GXNU YU000906, GXNU YU000908, and GXNU YU000909) is similar to holotype, paratype GXNU YU000907 has a lighter coloration, three paratypes (GXNU YU000903, GXNU YU000905, and GXNU YU000910) have a brown coloration (Fig. 7). Most paratypes share same characteristic as the holotype, having a broad black stripe between the eyes, whereas GXNU YU000903 and GXNU YU000910 lack this stripe (Fig. 7). The femoral glands are distinct in the holotype and most paratypes, but they are indistinct in GXNU YU000906 (Fig. 8).

Advertisement call. The call description is based on recording of an individual calling in a stream, with the ambient air temperature at 20 °C. Each call consisted of 9 to 18 (mean 13.5 ± 6.4; n = 2) notes, with a dominant frequency of 1507–1550 Hz (mean 1528.5 ± 30.4; n = 2). Call duration was 3856.5–7895.7 ms (mean 5876.1 ± 2856.2; n = 2). Call interval was 72.7 s. Each note had a duration of 164.9–224.9 ms (mean 199.7 ± 17.2; n = 27), and the intervals between notes were 183.3–439.9 ms (mean 254.4 ± 59.6; n = 25). Distinct amplitude variations within each note showed an overall trend of increasing and then decreasing in intensity (Fig. 9).

Distribution and ecology. The new species is currently only known from Wantian Township, Lingui District, Guilin City, Guangxi Zhuang Autonomous Region, China. The species inhabits a stream within a subtropical evergreen broad-leaved forest. The water in the stream is shallow (Fig. 10). During early September, male individuals were discovered on or beneath rocks (Fig. 11), and advertisement calls were heard, indicating that the new species is in breeding season.

Comparisons. The new species can be distinguished from *Brachytarsophrys popei* by the shallow notching at the posterior end of the tongue (vs. deep notching), distinct canthus rostralis (vs. indistinct canthus rostralis), iris red (vs. iris brownish), head width about 1.5 times head length (vs. head width about 1.2 times head length), first finger equal in length to second (vs. first finger longer than second), inner metatarsal tubercle approximately equal to first toe (vs. inner metatarsal tubercle longer than first toe), and webbing formula I1 - 2II1 - 2III1½ - 3-IV3- - 1½V (vs. I1½ - 2II1½ - 3III2½ - 3⅔IV3⅔ - 2V) (Zhao et al. 2014; Li et al. 2020). Furthermore, the PCA

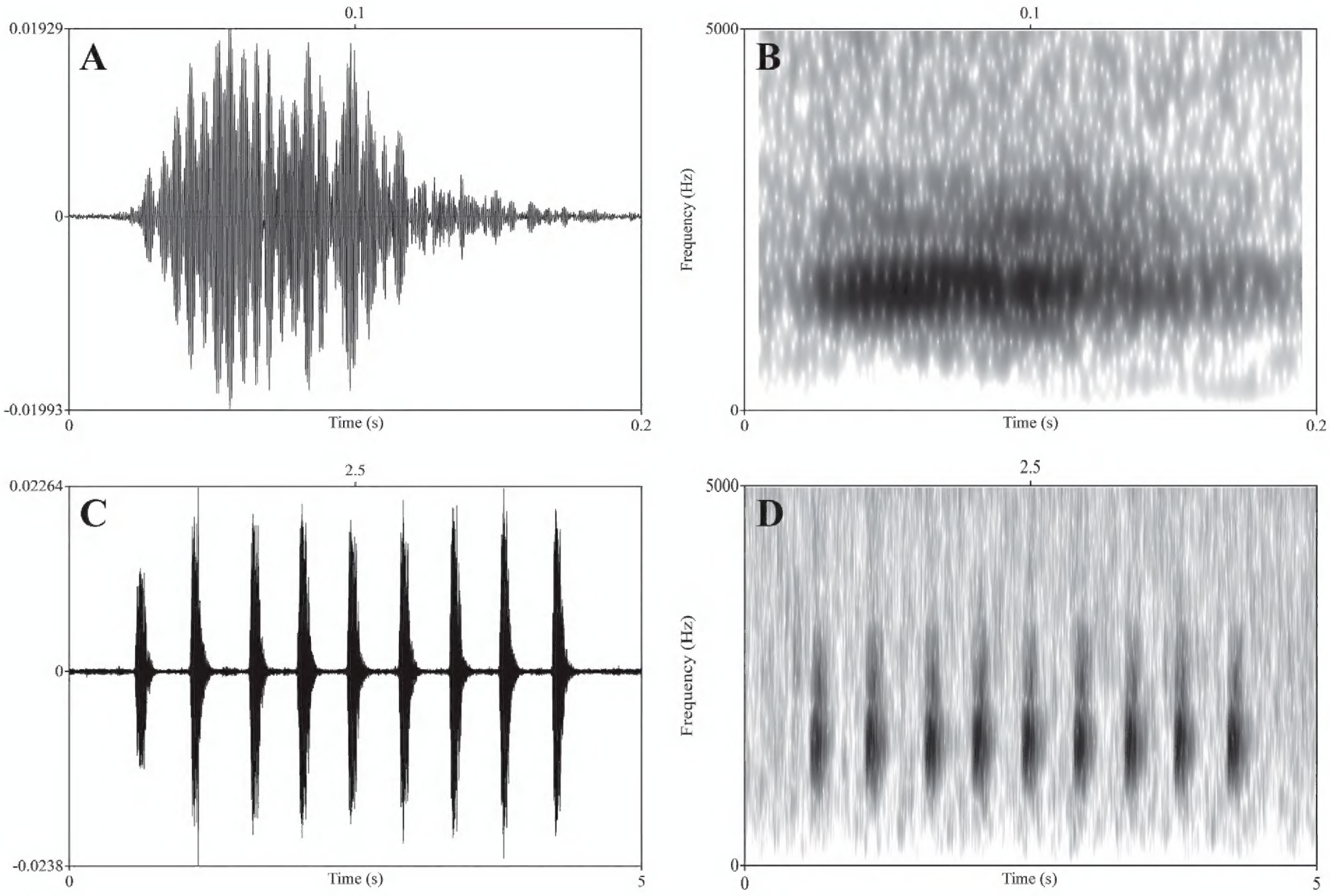

Figure 9. Visualization of advertisement calls of *Brachytarsophrys guilinensis* sp. nov. **A.** Waveform showing one note; **B.** Sonogram showing one note; **C.** Waveform showing 9 notes of one call; **D.** Sonogram showing 9 notes of one call (**A** and **B** are the same note, and **C** and **D** are the same call).

Figure 10. Microhabitat of *Brachytarsophrys guilinensis* sp. nov. at the type locality.

Figure 11. *Brachytarsophrys guilinensis* sp. nov. beneath rocks (Photo by Guohua Yu).

analysis suggested that the new species also differs from *B. popei* by a series of morphometric characters associated with head and limbs, such as shorter and narrower head, smaller eye diameter, shorter snout length, forearm length, and foot length.

The new species can be distinguished from *Brachytarsophrys orientalis* by distinct canthus rostralis (vs. indistinct canthus rostralis), iris red (vs. iris brownish), head width about 1.5 times head length (vs. head width about 1.2 times head length), first finger equal in length to second (vs. first finger longer than second), and webbing formula I1 - 2II1 - 2III1½ - 3-IV3- - 1½V (vs. I1½ - 2II1½ - 3III2½ - 4IV4 - 2V) (Li et al. 2020).

Brachytarsophrys guilinensis sp. nov. can be distinguished from other known congeners by the following characteristics: smaller body size, SVL of adult males 70–80 mm (vs. SVL of adult males 80–90 mm in *B. wenshanensis* and SVL of adult males > 90 mm in *B. carinense*, *B. intermedia*,

B. chuannanensis, *B. feae*, and *B. platyparietus*) (Boulenger 1889; Smith 1921; Taylor 1962; Rao and Yang 1997; Fei and Ye 2001; Fei et al. 2009; Li et al. 2020, 2022; He et al. 2024); head width about 1.5 times head length (vs. head width about 1.7 times head length in *B. qiannanensis* and head width about 1.2 times head length in *B. platyparietus* and *B. wenshanensis*) (Rao and Yang 1997; Fei et al. 2009; Li et al. 2020, 2022; He et al. 2024); male with nuptial pad on dorsal surface of the first and second fingers (vs. male with nuptial pad on dorsal surface of the first finger in *B. qiannanensis*, and male lacking nuptial pad in *B. wenshanensis*) (Li et al. 2022; He et al. 2024); tibiotarsal articulation reaching angle of mouth when hind limbs are extended forward alongside the body (vs. only reaching behind angle of the mouth in *B. feae*) (Boulenger 1887; Fei et al. 2009; Li et al. 2020); inner metatarsal tubercle approximately equal to first toe (vs. inner metatarsal tubercle longer than first toe in *B. feae*) (Boulenger 1887; Fei et al. 2009; Li et al. 2020); lateral fringes on toes wide (vs. lateral fringes on toes narrow in *B. wenshanensis*) (He et al. 2024). Moreover, the new species can be distinguished from *B. chuannanensis*, *B. feae*, *B. platyparietus*, *B. qiannanensis*, and *B. wenshanensis* by webbing formula I1 - 2II1 - 2III1½ - 3^{-}IV3^{-} - 1½V (vs. I1½ - 2^{++}II2 - 3^{++}III3 - 4IV4^{++} - 2½V in *B. chuannanensis*; I2 - 2^{++}II2^{-} - 3III2⅔ - 4IV4 - 2⅔V in *B. feae*; I1½ - 2^{+}II1½ - 3III2⅓ - 3⅔IV3⅔ - 2^{-}V in *B. platyparietus*; I1 - 2II2^{-} - 3^{-}III2½ - 4^{-}IV4 - 2^{-}V in *B. qiannanensis*; and I2^{-} - 2^{++}II2^{-} - 3^{++}III2½ - 4IV4^{+} - 2⅔V in *B. wenshanensis*) (Fei et al. 2009; Li et al. 2020, 2022; He et al. 2024).

Finally, *Brachytarsophrys guilinensis* sp. nov. differs from *B. qiannanensis* in dominant frequency, call duration, and call interval (Table 5).

Table 5. Acoustic comparisons between *Brachytarsophrys guilinensis* sp. nov. and *B. qiannanensis*.

	Dominant frequency (Hz)	Call durations (ms)	Call intervals (s)	Temperature (°C)	References
B. guilinensis sp. nov.	1507–1550	3856.5–7895.7	72.7	20	This study
B. qiannanensis	1640–2330	7690–11330	10.98–15.67	18	Li et al. 2022

Discussion

Guangxi is a part of the Indo-Burma Biodiversity Hotspot and is second only to Yunnan in amphibian species diversity in China (Tang et al. 2023). Including the new species described from northeastern Guangxi in this study, there are two *Brachytarsophrys* species recognized from Guangxi, China; the other one is *B. platyparietus*, which was confirmed from Mt. Jinzhong, Longlin County, in northwestern Guangxi (Li et al. 2020; AmphibiaChina 2025).

Rao and Yang (1997) considered that short-legged toads from Longsheng and Jinxiu counties belong to *Brachytarsophrys platyparietus*, which was once treated as a synonym of *B. carinensis* and was recorded from Huanjiang, Luocheng, Hezhou, Rongshui, Wuming, Longsheng, Jinxiu, Yulin, Xing'an, and Ziyuan counties in Guangxi (as *B. carinensis*; Fei et al. 2009; Mo et al. 2014). However, the taxonomy of *Brachytarsophrys* from northeastern Guangxi has never been confirmed by molecular study. In this study, we revealed that *Brachytarsophrys* from Wantian Township, Guilin, northeastern Guangxi, is neither *B. carinensis* nor *B. platyparietus* but represents a new species. Therefore, probably other records of *Brachytarsophrys* from adjacent localities in northeastern Guangxi (Xing'an, Longsheng, and Ziyuan counties; Fei et al. 2009) may also apply to *B. guilinensis* **sp. nov.**, pending additional data, and the records of *B. carinensis* or *B. platyparietus* from Jinxiu in central Guangxi and Yulin in southeastern Guangxi (Rao and Yang 1997; Fei et al. 2009) also need to be clarified. In addition, Fei et al. (2009, 2012) and Mo et al. (2014) recorded *B. feae* from Jingxi County in western Guangxi and Shangsi County in southern Guangxi. However, in China this species is currently recognized only from Yunnan (Li et al. 2020; AmphibiaChina 2025), so the taxonomy of records of *B. feae* from Jingxi and Shangsi also remains unclear. Overall, more studies are necessary to investigate the species diversity of the genus *Brachytarsophrys* in Guangxi, China.

It is interesting that *Brachytarsophrys popei* is composed of two distinct lineages, and they were placed into two taxonomic units by the analysis of species delimitation (Fig. 4). Considering that the phylogenetic relationship between these two lineages and *B. orientalis* was not well resolved (Fig. 3), and no distinct morphometric difference was found between these two lineages (Guangdong population vs. Yanling and Jiangxi populations; Fig. 2) in this study, future studies should incorporate more molecular and morphological data to clarify the phylogeny and taxonomy of them.

Including the new species *Brachytarsophrys guilinensis* sp. nov., the genus *Brachytarsophrys* now comprises ten recognized species. Previous studies have supported that this genus can be divided into two major lineages (e.g., Li et al. 2022; Lyu et al. 2023; He et al. 2024): one is distributed in the southern part of the Indochinese Peninsula and contains *B. carinense* and *B. intermedia* (*B. carinense* group; Lyu et al. 2023), and one contains the other species and is distributed in southern China (*B. orientalis* group; Lyu et al. 2023). This study confirms the existence of two major groups within this genus and indicates that the new species belongs to the *B. orientalis* group.

Acknowledgments

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providing tissue samples of *B. popei*. Thanks also go to Xinmin Zhang for his assistance in sample collection. This work was supported by the National Natural Science Foundation of China (32460128), the Guangxi Natural Science Foundation Project (2022GXNSFAA035526), and the Key Laboratory of Ecology of Rare and Endangered Species and Environmental Protection (Guangxi Normal University), Ministry of Education (ERESEP2022Z04).

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Supplementary material 1

Genetic distances between members of *Brachytarsophrys* (uncorrected p-distance) in 16S rRNA sequences

Authors: Xiangjian Wu, Yuanqiang Pan, Wei Xiao, Ju Chen, Guohua Yu, Xinkui Wei

Data type: xls

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Link: https://doi.org/10.3897/zse.101.157834.suppl1

Supplementary material 2

Genetic distances between members of *Brachytarsophrys* (uncorrected p-distance) in COI sequences

Authors: Xiangjian Wu, Yuanqiang Pan, Wei Xiao, Ju Chen, Guohua Yu, Xinkui Wei

Data type: xls

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Supplementary material 3

Genetic distances between members of *Brachytarsophrys* (uncorrected p-distance) in Cytb sequences

Authors: Xiangjian Wu, Yuanqiang Pan, Wei Xiao, Ju Chen, Guohua Yu, Xinkui Wei

Data type: xls

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